Liquid Chromatographic Method for the Determination of Lutein in Milk and Pediatric Formulas

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ABSTRACT

A simple, rapid method has been developed for routine compliance testing of lutein in pediatric formulas. Following a mild saponification procedure (70 °C for 10 min) demonstrated to be benign to lutein, lipophilic components were partitioned by a single extraction into hexane:diisopropyl ether (75:25 v/v) and concentrated in mobile phase. Baseline chromatographic separation of lutein and zeaxanthin (internal standard) was achieved using a C30 column and isocratic elution with methanol:dichloromethane (70:30 v/v). Lutein concentration was quantitated against zeaxanthin, which corrected for incomplete extraction recovery. Performance parameters assessed included recovery (101-108%) and repeatability (2.2% RSD). The method was applied to the analysis of lutein supplemented pediatric formulas, unsupplemented milk powders, bovine and human milk.

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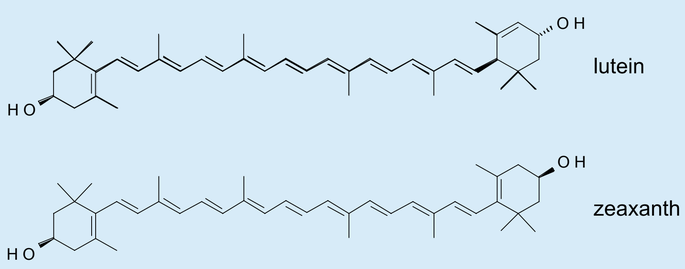
INTRODUCTION

Carotenoids confer remarkably diverse physiological functions in photosynthesis, pro-vitamin A activity, antioxidant status, immune function, and cell differentiation. Since lutein and zeaxanthin are the only dietary carotenoids in the macular pigment, they have received increasing attention for their dietary potential to reduce age-related macular degeneration. Further, lutein and zeaxanthin are both considered to be protective in neonatal retinal epithelium, and infant formulas are therefore increasingly supplemented with lutein to more closely resemble human breast milk.



ANALYTICAL TECHNIQUE

Sample Preparation

- Dissolve 1.0 g powder in 10 mL of ethanolic pyrogallol (1% w/v) in a 60 mL boiling tube, add 1.00mL aliquot of zeaxanthin intermediate standard (0.9 μg mL^{-1}) and 2 mL of potassium hydroxide (50% w/v), mix, then incubate at 70ºC for 10 mins in a water bath
- Cool, add 20 mL of hexane:diisopropyl ether (75:25 v/v), shake for 5 mins, wash with 20 mL water, mix, then centrifuge for 10 min at 200 x g
- Transfer 1 mL of the upper solvent layer to an HPLC vial, blow to dryness, and reconstitute with 1 mL mobile phase ready for analysis.

Chromatographic conditions

Column: YMC Carotenoid C30, 250 x 4.6 mm, 3 µm (Waters)
Mobile Phase: Methanol:dichloromethane (70:30 v/v) run isocratically at a
Å g-b jYl] g^ (&- eD eaf
Detection: Photo-diode array (370-600 nm) with quantitation at 450 nm
Quanititation: Internal standard technique (zeaxanthin)
Injection: 50 µL

Method performance parameters

Analyte	Range (ng mL^{-1})	Linear regression	r^2	MDL (µg $100g^{-1}$)	RSD_r [b] (%)	RSD_{iR} [c] (%)	$HORRAT_r$	Recovery [d] (%)	Recovery [e] (%)
Lutein	15.7–470.9	y = 1414.4x – 2377.3	0.9999	1.4	2.2	3.2	0.3	101-108	95
Zeaxanthin	20.6–618.5	y = 1367.7x – 5936.1	0.9997	–	–	–		–	–

[a] Method detection limit: determined from *n* replicates at or near the expected detection limit, MDL = $t_{(n-1, 1-a)}$ x sd, where $n = 7$ and $\alpha = 0.05$
[b] Relative standard deviation repeatability: RSD_r = sd/mean x 100, $n = 6$
[c] Relative standard deviation intermediate reproducibility: RSD_{IR} = sd/mean x 100, $n = 18$ (6 samples tested on 3 different days)
[d] Mean recovery of lutein spiked blank skim-milk samples (3 levels each in triplicate)
[e] Mean recovery of two independent samples by the described method, expressed as a percentage of total extractable lutein (measured as the aggregate of 4 pooled extractions quantitated against lutein external standard).

DISCUSSION

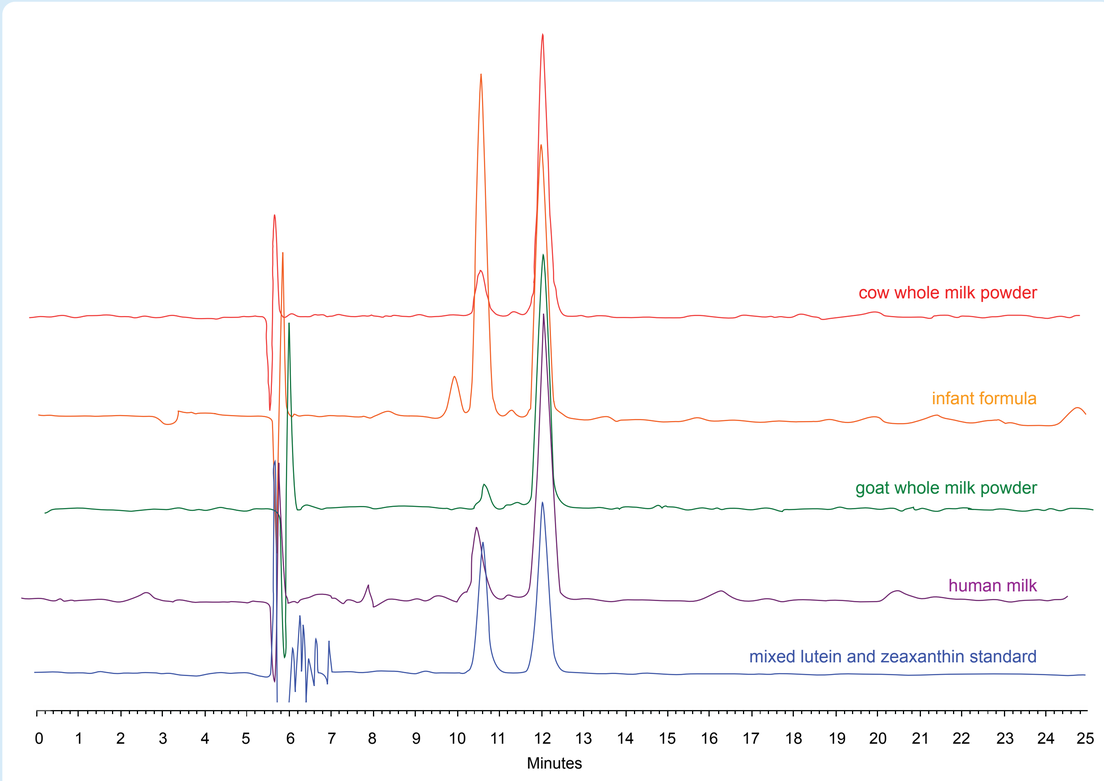
The method presently described was developed primarily for the compliance testing of lutein-supplemented pediatric formulas. It incorporates a mild alkaline hydrolysis and single extraction technique combined with an isocratic chromatographic separation.

=pljY[lagf j][gn]jq oYk]nYdmYl]\ ^gj Y fmeZ]j g^ eg\aÄ]\ fgf%hgdY found that all required at least three extractions for quantitative recovery. This was not unexpected in view of the relative high polarity of lutein compared to the parent carotene. Therefore, to simplify the analysis by retaining a single extraction, quantitation by an internal standard technique was necessary.

Zeaxanthin was chosen as internal standard since it is structural similar to lutein and is resolved form other peaks under the described chromatographic conditions. The applicability of this method to each matrix was initially assessed by analysing each sample type in the absence of internal standard to reveal the potential contribution of endogenous zeaxanthin. In goat whole milk powder and pediatric formulas, there were negligible amounts of endogenous zeaxanthin. While human and bovine milks showed trace levels of endogenous zeaxanthin, the quantitation of lutein was not ka_faÄ[YfIdq]^^][l]\ _an]f l`] j]dYlan]dq `a_` d]n]d g^ afl]jfYd klY

The impact of high temperature alkaline hydrolysis on lutein and zeaxanthin recovery was assessed \aj][ldq$ oal`gml kgdn]fl ]pljY[lagf$ Yf\ dgkk]k \mjaf_ l`] \]k[jaZ]\ [gfÄje]\ Yk YfYdqla[Yddq afka_faÄ[Yfl ^gj Zgl` [Yjgl]fga\k&

This is a rapid and robust method for the analysis of lutein in pediatric formulas, as well as being suitable for estimation of endogenous lutein in milk.



CONCLUSIONS

- **A simple reversed phase HPLC technique for lutein analysis is described.**
- **Method applicable for compliance testing of lutein-supplemented pediatric formulas, and for estimation of endogenous lutein in milk.**
- **Endogenous lutein levels measured in reconstituted infant formulas and bovine whole milk powders ranged from 1.0 to 5.8 µg 100 mL^{-1}.**
- **Comparable levels of lutein measured in fluid bovine and human milks at 6.0 and 4.3 µg 100 mL^{-1}, respectively.**

ACKNOWLEDGMENTS

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